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Research Note

Characterization of Yolk Protein in *Portunus pelagicus* (Linnaeus, 1758)

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Vitellogenesis in crustaceans is an important physiological process associated with reproduction and is characterized by synthesis of vitellogenin, its subsequent processing and accumulation within the developing oocytes. The major eggyolk protein vitellin is a lipoglycoprotein formed by the cleavage of the female-specific protein vitellogenin (Byrne *et al.*, 1989). Vitellogenin circulates in the haemolymph, while vitellin accumulates in the developing oocytes during the process of vitellogenesis. Vitellogenin and vitellin are known to be larger molecular compounds while the complexity and heterogeneity of their structures among crustacean species are still unclear. However, the efforts have mostly been focused on determination of their components and moiety. In the present study, an attempt is made to characterize the yolk protein in the ovary of the blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758), which is one of the dominant species of the marine crab resources of India and also a candidate species for aquaculture.

Earlier studies have shown that yolk proteins generally contain carbohydrates, phospholipids and carotenoid components (Chen *et al.*, 1999). The main protein fraction of the crustacean vitellus, the high density lipoglycoprotein is frequently associated with carotenoid pigments and is usually referred to as lipovitellin. Crab lipovitellin is 40 to 50% lipid, most of which is phospholipid (Lee & Walker, 1995). The composition of yolk varies from species to species and at times even among individuals, depending on diet. It mainly consists of water, protein and lipids. The protein provides the basic structural material needed for tissue build-up and the lipids serve as the major fuel (Adiyodi & Subramoniam, 1983). In addition to lipovitellin, the yolk may contain non-conjugated simple protein fractions and some glycoproteins. Earlier workers have reported ovary, hepatopancreas, adipose tissue and haemocytes to be some of the sites for vitellin synthesis in decapods (Chen *et al.*, 1999; Lee & Watson, 1995). Diversity in vitellin components as revealed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) has been reported in several crustacean species. In brachyurans, the numbers of vitellin subunits estimated are between two and four (Lee & Watson, 1995). The present study was conducted to characterize the yolk protein in the ovary of the blue swimmer crab *potunus pelagicus* (Linnaeus 1798), which is an important step in understanding the vitellogenesis and reproductive biology.

Wild specimens of female *Portunus pelagicus* of different sizes were collected from gillnetters operating in the Gulf of Mannar, off Mandapam (9°11′ N, 78°56′ E) during October-November 2006. The animals were transported live to the laboratory and maintained in 1 tonne FRP tanks with

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continuous aeration for a conditioning period of 24 hours. Feeding was done with raw squid and clam meat (1:1) *ad libitum* daily. Water was exchanged daily at the rate of 50%. Physicochemical parameters such as temperature, salinity and pH were maintained at 28.0±1.0°C, 34 ppt and 8.1±0.4 respectively. The animals were dissected live and the reproductive stages were identified based on the macroscopic characteristics according to Ryan (1967).

The ovarian tissues were subjected to Polyacrylamide gel electrophoresis (PAGE) under native conditions for understanding the nature of the egg yolk protein present. Ovarian homogenates for PAGE analysis was prepared according to Qiu *et al.* (1997). The ovary samples in different maturity stages *viz.* immature, early maturing, late maturing, mature and spent were collected and washed in ice cold 100 mM phosphate buffer containing 0.001% phenyl methyl sulfonyl fluoride (PMSF), which acted as a protease inhibitor. The samples were then stored at -20°C until study. The frozen ovaries were thawed later and homogenized in three volumes of the same buffer using a hand held homogenizer and centrifuged at 10,000 rpm for 15 minutes at 4°C. The floating layer of fat and the precipitate from the ovaries were carefully removed. As the fat content was high, the process had to be repeated thrice to get a clear supernatant. The supernatant, containing the ovarian protein was stored at -80°C until use. Discontinuous PAGE (Davis, 1964) was carried out with 5% stacking gel. From the standardization trials, 7.5% gel was found to give a better resolution and separation. Confirmation of the protein isolated as vitellin was done by selective staining of proteins in the Native gel as per Covens *et al.* (1988). Premise of staining is the knowledge that yolk protein is a lipo-glyco-caroteno-protein possessing a calcium moiety. The gels were stained with reagents such as Sudan black B, Periodic acid Schiff's reagent (PAS) and Alizarin red S for detecting the presence of lipid, carbohydrate and calcium, respectively. The subunit compositions of the purified vitellin were determined using SDS-PAGE according to Laemmli (1970) using 10% separating gel and 4% stacking gel. After electrophoresis, the gels were stained with Coomassie brilliant blue and subsequently destained in methanol acetic acid. Standard protein markers of known molecular weight (Sigma) such as phosphorylase, 97.5 kDa; bovine serum albumin, 66 kDa; ovalbumin, 43 kDa and carbonic anhydrase, 29 kDa were loaded to find out the weight of the separated protein units. The logarithm of molecular mass markers was plotted against electrophoretic mobility and linear regression was used to calculate the relative molecular mass of the samples.

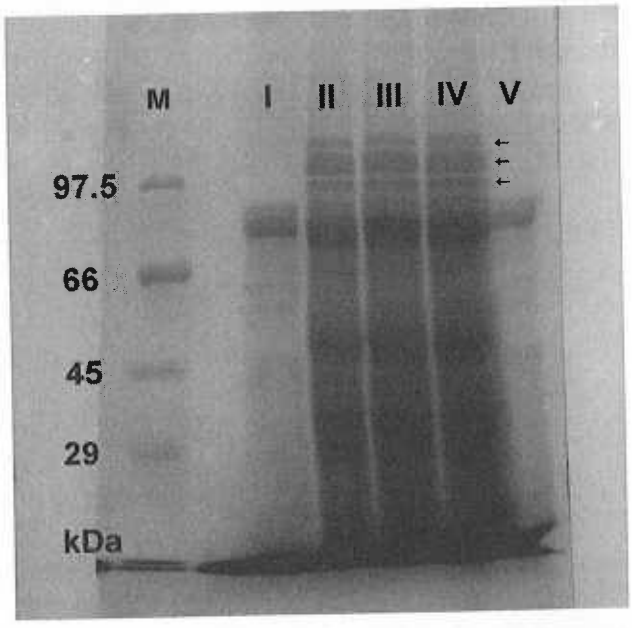

Fig. 1. SDS - PAGE of vitellin in Portunus pelagicus showing different subunits. (M = molecular markers, Stages I = immature, II = early maturing, III = late maturing, IV = mature, V = spent)

From an array of many ovarian proteins, a high molecular weight fraction was identified as the yolk protein vitellin by selective staining. The protein was not expressed in immature and spent ovaries in the case of *Metapenaeus monoceros* as reported by Abraham (2005). Similarly, in immature females of *Libia emarginata* lipoprotein was lacking (Hinsch & Cone, 1969). Other protein bands present in the Native PAGE gel did

not show positive results in the selective staining confirmatory test. The yolk proteins occur as large molecular weight proteins of 300-500 kDa with carotenoid, sugar/carbohydrate, calcium and lipid moieties attached during biosynthesis. These characters distinguish the yolk proteins from the other proteins like haemocyanin circulating in the haemolymph (Adiyodi & Subramoniam, 1983). Vitellogenins and vitellins are of low electrophoretic mobility.

In the present study, the selective staining reactions with different stains revealed that the vitellin in the oocytes of *P. pelagicus* is a lipo-glyco-caroteno-protein with calcium moiety. The absence of vitellin in the immature and spent stages as observed in the present study could be possibly because it is synthesized only in a negligible quantity in the immature stage and is present only in traces in the spent ovary, as suggested by Adiyodi & Subramoniam (1983). When subjected to SDS-PAGE, the high molecular weight protein portion got split into three subunits of molecular weights 103.5, 98.7 and 94.2 kDa (Fig. 1). The molecular weight of the associated vitellin was estimated to be 296.4 kDa. This is in agreement to Yang *et al.* (2005) who demonstrated three major polypeptides of molecular weights 102, 100 and 85 kDa by SDS-PAGE and Western blotting in the ovary of the mature crab *P. trituberculatus*. Similarly, three subunits of molecular masses 185, 100 and 84 kDa where identified from the ovary of *Cancer antennarius* (Spaziani *et al.*, 1995). According to Chen *et al.* (2004), vitellogenin had a native molecular mass of 520 kDa and the denaturing SDS-PAGE revealed two subunits of 97 and 74 kDa in the Chinese mitten handed crab *Eriocheir sinensis*. In decapods, the molecular weight of vitellin is found to range from 283 kDa to over 600 kDa (Tsukimura, 2001). Wallace *et al.* (1967) who characterized lipovitellin of several species of crustaceans estimated its average molecular weight to be 350 kDa. The fragmentation of larger molecular yolk proteins into subunits has been reported in many decapods like *Litopenaeus vannamei* and *Penaeus semisulcatus* (Tom *et al.*, 1992). In the land crab *Potamon potamios*, three major vitellin peptides of 115, 105 and 85 kDa have been reported by Pateraki & Stratakis (2000).

The present study on *P. pelagicus* applying SDS-PAGE revealed three subunits of vitellin, ranging in molecular weights from 94.2 kDa to 103.5 kDa, which are agreement with the observations made by earlier workers in other crustaceans. Characterization of yolk protein is an important step in understanding the process of vitellogenesis and reproductive biology of this economically important species.

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